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Comparison of solid phase- and liquid/liquid-extraction for the purification of hair extract prior to multi-class pesticides analysis

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ABSTRACT

The present study focuses on the influence of a purification step – after extraction of pesticides from hair and before analysis of the extract – on the sensitivity of analytical methods including compounds from different chemical classes (both parent and metabolites). Sixty-seven pesticides and metabolites from different chemical classes were tested here: organochlorines, organophosphates, carbamates, pyrethroids, ureas, azoles, phenylpyrazoles and neonicotinoids. Two gas chromatography-negative chemical ionization–tandem mass spectrometry methods and one based on ultra-performance liquid chromatography–electrospray tandem mass spectrometry were used. Seven solid-phase extraction cartridges: C18, S-DVB, PS-DVB, GCB, GCB/PSA, SAX/PSA and Florisil/PSA were tested and compared to more classical liquid–liquid extraction procedures using ethyl acetate, hexane and dichloromethane. Although LLE allowed obtaining good results for some compounds, on the whole, SPE clearly provided better recovery for the majority of the pesticide residues tested in the present study. GCB/PSA was clearly the best suited to non-polar compounds such as organochlorines, pyrethroids and organophosphates, with recovery ranging from 45.9% (diflufenican) to 117.1% (parathion methyl). For hydrophilic metabolites (*e.g.* dialkyl phosphates and other organophosphate metabolites, pyrethroid metabolites, phenols and carbamate metabolites), the best results were obtained with PS-DVB, with recovery ranged from 10.3% (malathion monocarboxylic acid) to 93.1% (para-nitrophenol). For hydrophilic parent pesticides (*e.g.* neonicotinoids, carbamates, azoles) and metabolites without nucleophilic functions, the best recovery was obtained with SAX/PSA, with recovery ranging from 52.1% (3-hydroxycarbofuran) to 100.9% (3,4-dichloroaniline). Solid phase extraction was found to be more suitable than the liquid–liquid extraction for pesticides and their metabolites determination in terms of number of extracted compounds and their recovery. Moreover, the use of solid phase extraction cartridges has enabled the reduction of the analytical background noise, resulting in better chromatographic separations.

Abbreviations: LLE, liquid–liquid extraction; SPE, solid phase extraction; GC–MS/MS, gas-chromatography tandem (triple quadrupole) mass spectrometry; LC–MS/MS, liquid-chromatography tandem (triple quadrupole) mass spectrometry; C18, octadecyl bonded silica sorbent; S-DVB, styrene divinylbenzene sorbent; PS-DVB, polystyrene divinylbenzene resin; GCB, graphitized carbon black sorbent; SAX, strong anion exchange sorbent; PSA, primary and secondary amines sorbent; Na_2SO_4, sodium sulfate; K_2CO_3, potassium carbonate; PFBBr, 2,3,4,5,6-pentafluorobenzylbromide; o,p'-DDE, 1,1-dichloro-2(o-chlorophenyl)-2-(p-chlorophenyl) ethylene; p,p′-DDE, 1,1-dichloro2,2-bis(p-chlorophenyl) ethylene; o,p′-DDD, 1,1-dichloro-2-(o-chlorophenyl)2-(p-chlorophenyl)ethane; p,p′-DDD, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane; o,p′-DDT, 1,1,1-trichloro-2,2-bis(o,p-chlorophenyl)ethane; p,p′-DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; α, β, γ, δ-HCH, α, β, γ, δ-hexachlorocyclohexane; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; DMDTP, dimethyldithiophosphate; DEP, diethylphosphate; DETP, diethylthiophosphate; DEDTP, diethyldithiophosphate; TCPy, 3,5,6-trichloro-2-pyridinol; TCPY, 2-isopropyl-4-methyl-6-hydroxypyrimidine; $ClCF_3CA$, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylic acid; Cl_2CA, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; 2-ClBA, 2-(4-chlorophenyl)-3-methylbutyric acid; Br_2CA, 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; PCP, pentachlorophenol; 2-IPP, 2-isopropxyphenol; PNP, para-nitrophenol; Malathion, CA malathion monocarboxylic acid.

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1. Introduction

The widespread use of pesticide in human surroundings has made exposure to these chemicals unavoidable. Pesticides are widely used in agriculture, but also for domestic use in homes and gardens, for roads and railways maintenance, in public indoor spaces and workplaces for pest control (*e.g.* common roach, ant, and termite). Forty-five percent of fruits, vegetables and cereals which are grown in the European Union contain pesticide residues [1].

Although some studies are already revealing risks for health (*e.g.* neurotoxicity, developmental effects, endocrine disruptor properties) associated with exposure to some common pesticides such as organophosphate (OP) or pyrethroids insecticides, a wide number of pesticides to which human is commonly exposed (*e.g.* herbicides, fungicides) has not been investigated yet with regard to possible exposure-associated effects [2,3]. In the same way, effects associated with multiple exposure remains poorly documented.

Exposure can be highlighted by the determination of pollutants in environmental matrices (air, water, soil, *etc.*) or in food, and subsequent assessment of the transfer to human. The effective entrance into the body is however estimated by the analysis of human biological matrices. To date, the most frequently used matrices for the biomonitoring of human exposure are blood and urine. In the case of pesticides, blood is generally used for the determination of parent compounds [4–8], whereas urine analysis is mostly performed for the determination of metabolites [4,9,10]. Over the last years, a growing interest has also been observed in hair analysis for the biomonitoring of environmental and occupational exposure to organic pollutants [2,11,12]. Hair, initially used for forensic and clinical purposes, is currently the most used "alternative matrix" for human biomonitoring [13]. On the one hand, the main advantage of hair analysis is the access to an extended window of detection, compared with blood and urine. On the other hand, as a solid matrix, hair requires more complex pre-analytical procedures (extraction and purification) than biological fluids.

A variety of extraction methods has previously been used for the analysis of pesticides in hair, including acidic hydrolysis, soxhlet extraction, and extraction with organic solvents of the analytes from the solid matrix [12,14]. Following acidic hydrolysis or soxhlet extraction, extracts were further purified prior to analysis, generally by liquid–liquid extraction (LLE) using ethyl acetate, cyclohexane, hexane: ethyl acetate, hexane:dichloromethane; or by solid phase extraction using glassy cartridges with acidic or alkaline silica, activated alumina, and florisil [14]. Later on, more gentle procedures were used for the extraction of chemicals that might be altered by the extraction conditions (*e.g.* organophosphates, carbamates, and pyrethroids) [12,15]: water extraction with sonication or agitation at room temperature [16,17]; hexane extraction at room temperature [15]; incubation in ultrasonic bath and extraction with methanol at low temperature [18]; or acetonitrile incubation overnight at 40 °C under agitation [19].

Following extraction, purification may also be necessary to remove impurities which are massively extracted from hair along with target analytes and are responsible for significant analytical background noise. Purification is performed on the basis of physicochemical properties differences between the target compound and impurities. This step, which might already be challenging for a single target compound, is likely to become quite problematic for multi-residue methods focusing on analytes from different chemical classes [19]. Nevertheless, the increasing awareness of the importance of taking into account multiple exposure (*i.e.* simultaneous exposure to several different chemicals) in the study of exposure-associated health effects makes it relevant to investigate analytical developments allowing for multi-residue analysis.

In the case of pesticide residue analysis in urine, the most applied clean-up techniques are liquid–liquid extraction (LLE) used for many years as routine technique [20,21] and solid phase extraction (SPE), mainly using C18 [22–24] or polymeric sorbents (*e.g.* poly-methyloctadecylsiloxane, polystyrene divinylbenzene resin) [24,25]. Nevertheless, to the best of our knowledge, unlike urine, no study dealing with the application of LLE and SPE prior to multi-residue analysis of pesticides in human hair has been published to date.

Therefore, the present study focuses on the influence of a purification step – after extraction of pesticides from hair and before analysis of the extract – on the sensitivity of an analytical method including compounds from different chemical classes. Sixty-seven pesticide residues from different classes were analyzed: organochlorines, organophosphates, carbamates, pyrethroids, ureas, azoles, phenylpyrazoles and neonicotinoids. Pesticide residues, including both parent compounds and metabolites, were analyzed in hair extracts by gas- or liquid-chromatography tandem (triple quadrupole) mass spectrometry (GC– and LC–MS/MS). Six solid-phase extraction cartridges commercially available and one homemade were tested and compared with each other and to more classical liquid–liquid extraction procedures, using ethyl acetate, hexane and dichloromethane. The SPE cartridges evaluated in this study were selected on the basis of their retention mechanism: 4 reversed-phase cartridges compatible with aqueous solutions; (a) C18, a widely used octadecyl bonded silica sorbent having the broadest spectrum of retention; (b) S-DVB, a non-polar styrene divinylbenzene sorbent adapted to small molecules; (c) PS-DVB, a polystyrene divinylbenzene resin used to retain hydrophobic compounds with some hydrophilic functionality; (d) GCB, a graphitized carbon black sorbent for organic polar and non-polar compounds; and 3 anion-exchange cartridges compatible with organic solutions: (a) SAX/PSA, a dual layer cartridge that contains a strong anion exchange (SAX) quaternary amine, Cl-counter-ion, and an ethylenediamine-N-propyl phase that contains both primary and secondary amines (PSA) sorbents; (b) GCB/PSA, a dual layer cartridge that contains both GCB and PSA sorbents; and (c) Florisil/PSA, a triple layer cartridge that contains a magnesia-loaded silica gel (Florisil), PSA and Na_2SO_4 (drying layer).

2. Materials and methods

2.1. Chemicals and reagents

Dinotefuran; 1,1-dichloro2,2-bis(p-chlorophenyl) ethylene (p,p′-DDE); 1,1,1-trichloro-2,2-bis(o,p-chlorophenyl)ethane (o,p′-DDT); 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p′-DDT); 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPy), dimethylphosphate (DMP); diethylthiophosphate (DETP); diethyldithiophosphate (DEDTP) and folpet analytical standards were obtained from Sigma–Aldrich (Bornem, Belgium); diethylphosphate (DEP), dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DMDTP) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA); the other pesticide standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) (the complete list of standards is provided in Tables 1–3). Individual stock solutions at 1 g L^{-1} of all standards were prepared by exact weighing of powder or liquid, and dissolution in acetonitrile. A working solution containing the 67 targeted compounds at 1 mg L^{-1} in acetonitrile was prepared. Acetonitrile, methanol, ethyl acetate, acetone and hexane were supplied by Biosolve (Valkenswaard, Netherlands). Sodium sulfate (Na_2SO_4), potassium carbonate (K_2CO_3) and ammonia were purchased from Merck (Darmstadt, Germany). Bond Elut C18, 500 mg C18, 3 mL; Bond Elut Plexa, 60 mg PS-DVB, 1 mL; and Bondesil-PSA were obtained from Agilent Technologies (Santa Clara, CA, USA). The derivative agent 2,3,4,5,6-pentafluorobenzylbromide (PFBBr) and